EXPERIMENTAL STUDIES IN THE STEROIDS

By

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EXPERIMENTAL STUDIES IN THE STEROIDS. THE OXIDATION OF METHYL 3(α)-ACETOXY-11-KETO-12-BROMOCHOLANATE*

BY DAVID M. CLARK,† NORMAN G. BRINK,‡ AND EVERETT S. WALLIS

(From the Frick Chemical Laboratory, Princeton University, Princeton)

(Received for publication, September 8, 1945)

An important intermediate in the synthesis of Compound E (I) is a compound whose structure is that indicated in formula II. This latter compound (m.p. 162–163°) has already been prepared by Sarett (1) by step by step degradation methods. In this paper we wish to describe certain oxidation experiments by means of which we have been able to remove the cholanic acid side chain in one operation. For this purpose[1] methyl 3(α)-acetoxy-11-keto-12-bromocholanate was prepared by the method of Reich and Reichstein (2) as modified by Sarett (1).

In our hands this compound melted at 176–178°. When it was submitted to oxidation by a method similar to that of Ruzicka and Goldberg (3) and the products of the oxidation worked up in such a manner as to remove the neutral ketonic material free from bromine, there was isolated in small yields by chromatographic methods Compound II, m.p. 158–160°.

EXPERIMENTAL

10 gm. of methyl 3(α)-acetoxy-11-keto-12-bromocholanate, m.p. 176–178°, were dissolved in 355 cc. of glacial acetic acid and to the solution, which was kept at 85–90°, there was added dropwise over a period of 4 hours, with stirring, a solution of 14.4 gm. of chromic acid dissolved in 14.4 cc. of water and 46.6 cc. of glacial acetic acid. After the addition was completed, the mixture was stirred for another 4 hours at a temperature of 85–90°. To the solution 45 cc. of methyl alcohol were then added and the contents were evaporated to a small volume (75 to 100 cc.) under diminished pressure. Water was then added and the material was ex-

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Princeton University.

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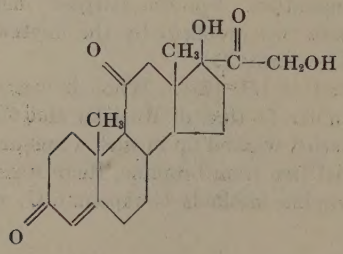
[1] This particular compound was chosen because it was our belief that the introduction of bromine to the 12 position α to the ketone group would sufficiently stabilize Ring C so that during the course of oxidation the ring would not be ruptured.

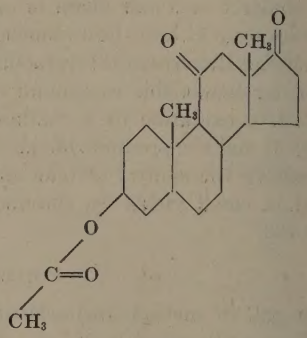
tracted with ether. The ether layer was washed with a 10 per cent solution of potassium carbonate several times to remove acidic materials. It was then dried and the ether evaporated completely on a steam bath. A white crystalline residue was obtained (weight, 5.2 gm.).

This residue was taken up in 200 cc. of acetic acid and debrominated in the usual manner with zinc dust. After debromination was complete, the material was poured into water and extracted with ether. On working up the ether-soluble product, a pale yellow viscous oil (3.78 gm.) was obtained.

Removal of non-ketonic neutral material was effected in the following manner. The pale yellow oil described above was taken up in 200 cc. of benzene and the solution evaporated to dryness. Methyl alcohol (14 cc.) was added and a white crystalline material separated. This was dissolved



(I)



(II)

under a reflux and to the solution were added 1.75 gm. of Girard's Reagent T and 1.4 cc. of glacial acetic acid. The refluxing was continued for 1 hour. After cooling, 72 cc. of ice water were added, and, with shaking in an ice bath, 140 cc. of 0.135 N NaOH (ice-cold) were added in small portions. The contents (still acidic) were shaken for an additional 10 minutes and then extracted with ether. The ethereal solution was washed and worked up in the usual manner. Weight of the non-ketonic neutral fraction, 3.51 gm.

The aqueous solution from the above experiment was treated with 3.67 cc. of 6 N H_2SO_4, calculated to bring the pH to 2.9 to 3.0. After about 45 seconds it was extracted with ether and the ether layer worked up in the usual manner. A crude crystalline product (50 mg.), of melting point 125–128°, was obtained (melting point of methyl 3(α)-acetoxy-11-keto-cholanate 130–132°).

To the aqueous portion after the removal of the product described above there was added enough ice-cold sulfuric acid to bring the acidity of the solution to approximately 0.75 N in H_2SO_4. After standing overnight at room temperature it was extracted with ether and the ether-soluble fraction worked up. To the oily residue so obtained there were added 75 cc. of water and the contents of the flask were submitted to steam distillation in order to remove volatile ketonic material. The non-volatile residue (197 mg.) was taken up in 10 cc. of benzene, and the solution evaporated in order to free the material from traces of water. It was then reacetylated by treatment with 1.8 cc. of acetic anhydride and 2.0 cc. of pyridine at the temperature of the water bath. The reacetylated product was submitted to chromatographic analysis, and the chromatogram developed with a mixture of petroleum ether and diethyl ether. Two fractions were obtained. Fraction I was rechromatographed and then taken up in a small volume of ethyl acetate. Pentane was added and the solution was seeded with an authentic specimen of the 3(α)-acetoxy-11,17-diketone prepared by Sarett (1). Crystalline rosettes soon formed and after several days standing were filtered and recrystallized. The product so obtained (10 mg.) melted at 158–160° and with an authentic specimen furnished us by Dr. Sarett gave no depression of melting point.

SUMMARY

The oxidation of methyl 3(α)-acetoxy-11-keto-12-bromocholanate with chromic acid has been studied. Small yields of 3(α)-acetoxycholandione-11,17 have been isolated, m.p. 158–160°, identical with the product of melting point 159–160° prepared by Sarett by step by step degradation methods.

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THE BROMINE DEGRADATION OF THE SILVER SALTS OF BILE ACIDS AND RELATED COMPOUNDS*

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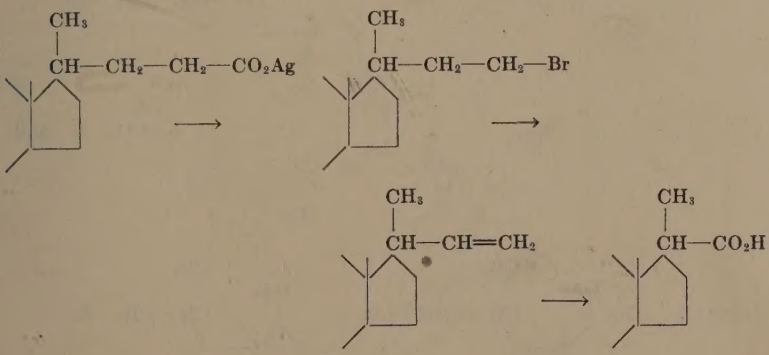
(Received for publication, September 8, 1945)

The reaction of bromine with the silver salt of a carboxylic acid to yield an alkyl bromide containing 1 less carbon atom

$$RCO_2Ag + Br_2 \rightarrow R{\cdot}Br + CO_2 + AgBr$$

has recently been studied by Hunsdiecker and Hunsdiecker (1).[1] In the present paper are reported the results obtained by application of this reaction to some bile acids and related compounds.



At the beginning of this work we had three principal objectives in mind. First, we wished to study the possibility of developing a method of degrading the bile acid side chain 2 carbon atoms at a time. Such a method would involve conversion of the silver salt of a bile acid to a norcholanyl bromide, dehydrobromination to a Δ^{22}-norcholene, followed by ozonolysis leading to a bisnorcholanic acid. Secondly, we desired to use the alkyl bromides

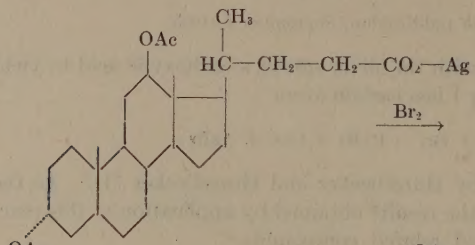
* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Princeton University.

† Present address, Research Laboratories, Merck and Company, Inc., Rahway, New Jersey.

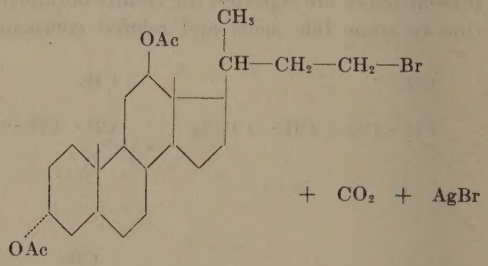
‡ At present a lieutenant in the United States Naval Reserve.

[1] In this article (1) will be found reference to the earlier literature on the reaction.

produced in this way as intermediates in the preparation of steroids containing a simple secondary alkyl group at C_{17}. Such molecules might well be better suited for Ruzicka type oxidations (*cf.* (2)) for the removal of the side chain than the carboxylic acids, esters, diphenylethylene, or diphenylethane derivatives which have been studied. Finally, we were



(I)



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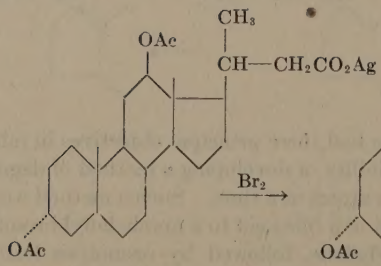

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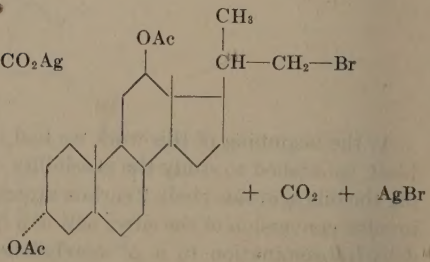
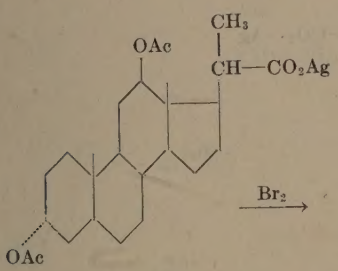


(IV)

particularly interested in preparing derivatives of the isomeric 20-bromopregnanes, since these, on dehydrobromination, would be expected to give $\Delta^{17,20}$ - or $\Delta^{20,21}$ - pregnenes, or both. The former on ozonolysis should yield an etiocholanone-17, and the latter an etiocholanecarboxylic acid. Both types of compounds are of importance as intermediates in the synthesis of the adrenal hormones.

Silver 3(α),12(β)-diacetoxycholanate (I) was prepared in essentially quantitative yield by treating a solution of the sodium salt of desoxycholic acid diacetate in dilute alcohol with an excess of aqueous silver nitrate solution. Upon addition of 1.06 moles of bromine to a suspension of the thoroughly dried silver salt in boiling carbon tetrachloride, vigorous carbon dioxide evolution was observed, and silver bromide appeared. When the reaction mixture was worked up, 3(α),12(β)-diacetoxy-23-bromonorcholane (II), m.p. 127.5–128.5°, $[\alpha]_D^{20} = +114°$, was obtained in 25 per cent yield. In like manner the silver salt of nordesoxycholic acid diacetate (III) was



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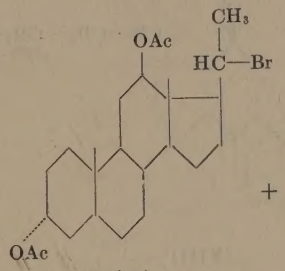

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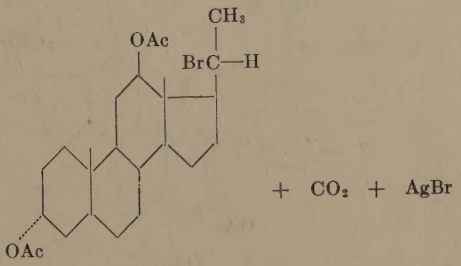
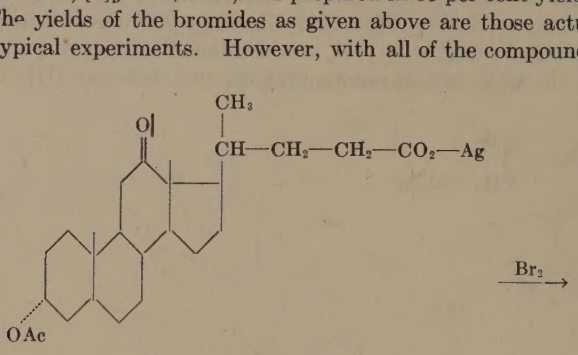


(VII)

converted to 3(α),12(β)-diacetoxy-22-bromobisnorcholane (IV), m.p. 144.5–145°, $[\alpha]_D^{20} = +107°$, in 40 per cent yield. When applied to the silver salt of bisnordesoxycholic acid diacetate (V), the bromine degradation led to a mixture of two isomeric bromides which was separated by chromatographic analysis and fractional crystallization. The more easily purified compound, designated 3(α),12(β)-diacetoxy-20(β)-bromopregnane (VI), crystallized in the form of small, hard needles, melting at 140–142°, $[\alpha]_D^{20} = +86°$. The isomeric 3(α),12(β)-diacetoxy-20(α)-bromopregnane (VII) appeared as fine, silky needles, m.p. 83–87°, $[\alpha]_D^{20} = +103°$. The yield of crystalline C_{20}-bromide mixture, before separation, was 65 per cent.

It was to be expected that a ketone group in the molecule would not interfere with the reaction, provided that no excess of bromine was used, since the silver salts react very rapidly with the halogen. This proved to be the case. From silver 3(α)-acetoxy-12-ketocholanate (VIII) the corresponding bromide, 3(α)-acetoxy-12-keto-23-bromonorcholane (IX), m.p. 209.5–211°, $[\alpha]_D^{20} = +126°$, was prepared in 60 per cent yield.

The yields of the bromides as given above are those actually obtained in typical experiments. However, with all of the compounds except des-



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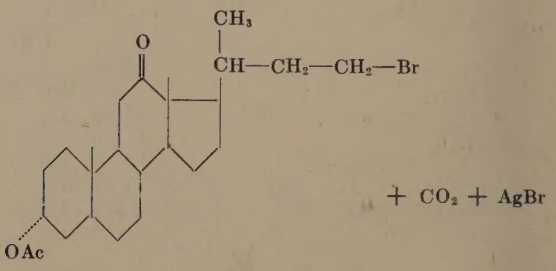


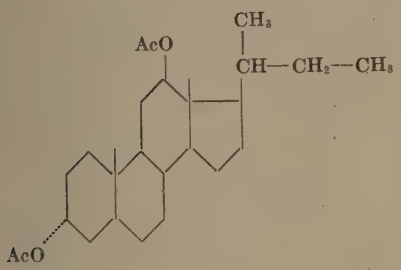
(IX)

oxycholic acid, varying amounts of the acetylated acids were recovered from the acid fractions. Correction for this recovery of starting material increases the yields cited above from 5 to 25 per cent.

With the alkyl bromide derivatives described above, reduction with zinc and acetic acid proceeded smoothly to give the corresponding steroids with alkyl side chains in yields which were uniformly over 90 per cent. The compounds so prepared are 3(α),12(β)-diacetoxynorcholane (X), m.p. 116.5–117°, $[\alpha]_D^{20} = +118°$; 3(α),12(β)-diacetoxybisnorcholane (XI), m.p. 115–116°, $[\alpha]_D^{20} = +104°$; 3(α),12(β)-diacetoxypregnane (XII),

m.p. 158–158.5°, $[\alpha]_D^{20} = +112°$; and 3(α)-acetoxy-12-ketonorcholane (XIII), m.p. 158.5–159.5°, $[\alpha]_D^{20} = +119°$.

Attempts to remove hydrogen bromide from the two primary diacetoxy bromides (II and IV) were unsuccessful. When the bromides were refluxed with collidine, unchanged starting material was recovered; treatment with piperidine led to stable piperidinium complexes. In the case of 3(α),-12(β)-diacetoxy-23-bromonorcholane, treatment with sodium ethylate, followed by reacetylation, gave in very low yield a compound melting at



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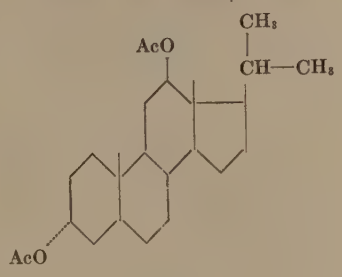

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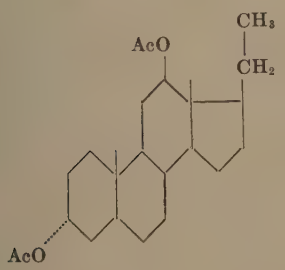

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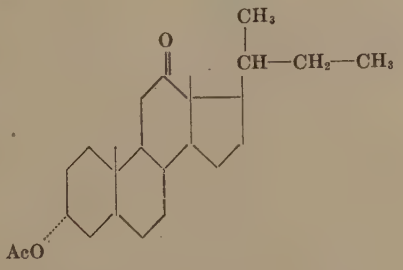


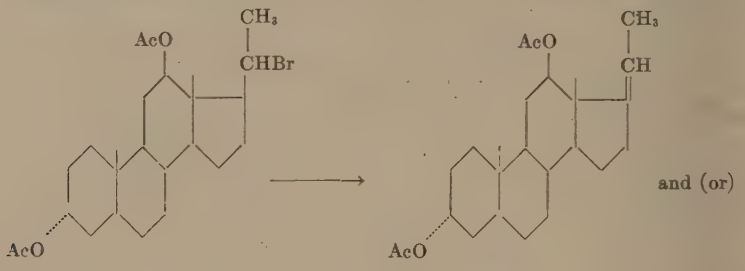
(XIII)

143–144°, $[\alpha]_D^{20} = +60°$, which on analysis proved to be acetoxy-23-ethoxy-norcholene. This apparently results from simultaneous ether formation and loss of a molecule of acetic acid from either Ring A or Ring C. The compound was not further investigated. Treatment of the primary bromides with alcoholic silver nitrate solution led to precipitation of silver bromide, but no definite organic product could be isolated.

The mixture of secondary C_{20}-bromides proved stable toward collidine, but when it was refluxed for 22 hours with piperidine, hydrogen bromide was removed and a product melting at 162–174° was isolated in 87 per cent yield by crystallization from methanol. After several recrystallizations,

material with a constant melting point of 176–178.5°, $[\alpha]_D^{20} = +112°$, was obtained. The product analyzed correctly for a diacetoxypregnene, and is presumably 3(α),12(β)-diacetoxy-$\Delta^{17,20}$-pregnene (XIV), 3(α),12(β)-diacetoxy-$\Delta^{20,21}$-pregnene (XV), or a mixture of both.

Ozonolysis of our material, or preferably of the related derivative or mixture prepared from 3(α)-acetoxy-11-keto-20-bromopregnane, should lead to a C_{17}-keto compound, an etio acid, or a readily separable mixture of the two.



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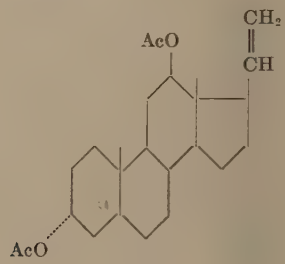

(XV)

EXPERIMENTAL[2]

3(α),12(β)-Diacetoxy-23-bromonorcholane (II)—Desoxycholic acid diacetate was prepared by heating 24.8 gm. of desoxycholic acid on the steam bath for 3 hours with 50 cc. of pyridine and 45 cc. of acetic anhydride. The product was worked up in the usual manner and the oil so obtained was dissolved in 300 cc. of 80 per cent ethyl alcohol. This solution was titrated to the phenolphthalein end-point with dilute aqueous sodium hydroxide, after which the pink color was just discharged by the dropwise addition of very dilute nitric acid.

[2] All melting points are uncorrected. Unless otherwise noted, rotations were taken in acetone in a 1 dm. tube (c = 0.8 to 1.3).

The solution was diluted to 800 cc. by addition of water, and a solution of 12.9 gm. (20 per cent excess) of silver nitrate in 150 cc. of water was added with swirling. About 500 cc. of water were added, and after thorough shaking the voluminous white precipitate was filtered off, washed three times with 20 per cent ethyl alcohol, and dried to constant weight, finally at 100° and 1 mm. over phosphorus pentoxide. The yield of light tan, powdery silver salt was 90 per cent.

To a suspension of 23.8 gm. of silver salt in 500 cc. of boiling anhydrous carbon tetrachloride were added over a period of about 10 seconds and with vigorous stirring 6.92 gm. (1.06 moles) of pure bromine in 147 cc. of carbon tetrachloride. Evolution of carbon dioxide and formation of silver bromide were observed immediately, and the color of bromine was discharged in the course of 4 minutes.

The stirring and refluxing were continued for an additional 10 minutes. The hot suspension was filtered to remove silver bromide and the solvent was taken off *in vacuo* below 40°. The light yellow residual oil was taken up in ether and the ethereal solution washed three times with 5 per cent sodium carbonate and three times with water. After the mixture had dried over sodium sulfate, the ether was removed to yield 10.6 gm. of a neutral, red oil. The oil was chromatographed on alumina. Fractions eluted by benzene and by benzene containing 1 per cent of ether crystallized on removal of the solvent and addition of methanol, m.p. 124.5–127°. Yield (combined fractions from several chromatographic analyses) 5.2 gm. (25 per cent). One recrystallization from methanol gave needles melting at 127.5–128.5°. The melting point was unchanged by further recrystallization. $[\alpha]_D^{20} = +114°$.

Analysis—$C_{27}H_{43}O_4Br$. Calculated. C 63.39, H 8.47, Br 15.62
Found. " 63.65, " 8.55, " 15.93

3(α),12(β)-Diacetoxy-22-bromobisnorcholane (IV)—Pure crystalline nordesoxycholic acid diacetate, m.p. 205–206°, was converted to the silver salt by the method described above. A 0.90 gm. portion of the salt, suspended in boiling carbon tetrachloride, was treated with 0.268 gm. (1.06 moles) of bromine in 1 cc. of carbon tetrachloride. After 10 minutes the reaction mixture was worked up as before, yielding a neutral fraction of 0.489 gm. and an acid fraction of 0.26 gm. The neutral fraction, upon being chromatographed on alumina, gave 0.30 gm. of needles, m.p. 143–144°. After one recrystallization from methanol, the product melted constantly at 144.5–145°; $[\alpha]_D^{20} = +107°$.

Analysis—$C_{26}H_{41}O_4Br$. Calculated. C 62.76, H 8.31, Br 16.06
Found. " 62.85, " 8.59, " 16.68

The acid fraction was crystallized and identified by melting point and mixed melting point as nordesoxycholic acid diacetate.

Isomeric 3(α),12(β)-Diacetoxy-20-bromopregnanes (VI and VII)—From 2.77 gm. of non-crystalline bisnordesoxycholic acid diacetate were prepared by the usual method 3.48 gm. (83 per cent) of its silver salt. This, suspended in carbon tetrachloride, was treated with bromine and worked up as previously indicated. The neutral fraction weighed 1.88 gm. and the acid fraction 1.11 gm.

Two-thirds (1.2 gm.) of the neutral fraction, dissolved in 20 cc. of benzene plus 80 cc. of petroleum ether, was chromatographed on 40 gm. of alumina. Elutions with mixtures of benzene and petroleum ether gave in the first fraction 953 mg. of fine, yellowish needles. The second fraction, when eluted by benzene containing a few per cent of ether, gave 101 mg. of coarse needles.

The material in the second fraction was twice recrystallized from methanol to give small, hard needles, m.p. 140–142°; $[\alpha]_D^{20} = +86°$ (designated as the 20(β)-bromo compound).

Analysis—$C_{25}H_{39}O_4Br$. Calculated, C 62.10, H 8.13; found, C 61.73, H 8.22

The first fraction on crystallization from methanol gave material melting at 103–106°. By addition of water to the mother liquor, 0.10 gm. of fine, silky needles, m.p. 83–87°, was obtained. The melting point remained constant on further recrystallization. $[\alpha]_D^{20} = +103°$. (This isomer has been designated the 20(α)-bromo compound.)

Analysis—$C_{25}H_{39}O_4Br$. Calculated, C 62.10, H 8.13; found, C 61.78, H 8.19

The material melting at 103–106°, mentioned above, had a specific rotation of $[\alpha]_D^{20} = +97°$. This intermediate rotation indicates that the first material obtained from Fraction I was a mixture of the two isomers.

3(α)-Acetoxy-12-keto-23-bromonorcholane (IX)—To 5.1 gm. of the silver salt of 3(α)-acetoxy-12-ketocholanic acid in 75 cc. of boiling carbon tetrachloride was added a solution of 1.52 gm. (1.00 mole) of bromine in 4.85 cc. of carbon tetrachloride. When, after filtration of the silver bromide, the solvent was removed *in vacuo*, the residue crystallized. It was broken up, filtered, and washed with ether. In this manner 1.61 gm. of crystals, m.p. 190–205°, were obtained. Recrystallization from chloroform-methanol gave 1.18 gm. of material, m.p. 205–208°. Two more recrystallizations from chloroform-methanol and two from dilute acetone gave a pure product, m.p. 209.5–211°. $[\alpha]_D^{20} = +126°$ (chloroform).

Analysis—$C_{25}H_{39}O_3Br$. Calculated. C 64.23, H 8.41, Br 17.1
Found. " 64.27, " 8.63, " 16.4

When reduced with zinc and acetic acid, 0.36 gm. of the bromo compound gave 0.29 gm. (97 per cent) of 3(α)-acetoxy-12-ketonorcholane (XIII), m.p. 157–159°. After two recrystallizations from methanol, the product melted at 158.5–159.5°. $[\alpha]_D^{20} = +119°$.

3(α),12(β)-Diacetoxynorcholane (*X*)—A portion of 3(α),12(β)-diacetoxy-23-bromonorcholane (4.48 gm.) was dissolved in about 150 cc. of glacial acetic acid and to the refluxing solution were added portionwise over a period of 15 minutes about 6 gm. of zinc dust. The hot solution was filtered directly into 5 volumes of cold water, and the residual zinc washed thoroughly with ether. The precipitated product was removed from the water by extraction with ether. The ethereal solution was washed with water, three times with 5 per cent sodium carbonate solution, and three times with water. The solution was dried over sodium sulfate and the ether removed to give 3.7 gm. of colorless oil. This was crystallized from methanol. The first crop of fine, long needles weighed 1.95 gm., m.p. 112–114°; the Beilstein test was negative. Three more crops of slightly less pure material were obtained by concentration of the mother liquor. Total yield 3.65 gm. (97 per cent). Several recrystallizations from methanol gave a material with a constant melting point of 116.5–117°. $[\alpha]_D^{20} = +118°$.

Analysis—$C_{27}H_{44}O_4$. Calculated, C 74.95, H 10.25; found, C 75.26, H 10.23

3(α),12(β)-Diacetoxybisnorcholane (*XI*)—1 gm. of 3(α),12(β)-diacetoxy-22-bromobisnorcholane, reduced with zinc and acetic acid exactly as above, gave 0.65 gm. of oil. This was crystallized from methanol to yield long, coarse needles, m.p. 105–107°. After four recrystallizations from methanol and three from dilute ethanol, pure material melting at 115–116° was obtained. $[\alpha]_D^{20} = +104°$.

Analysis—$C_{26}H_{42}O_4$. Calculated, C 74.60, H 10.11; found, C 74.46, H 10.36

3(α),12(β)-Diacetoxypregnane (*XII*)—A mixture of the two isomeric C_{20}-bromides was reduced with zinc and acetic acid in the usual manner. The total yield of crystalline product was 95 per cent. The material in the first crop melted at 156–158°. After two recrystallizations from methanol, pure crystals, m.p. 158–159.5°, $[\alpha]_D^{20} = +112°$, were obtained.

Analysis—$C_{25}H_{40}O_4$. Calculated, 74.21, H 9.97; found, C 74.50, H 10.18

Attempted Dehydrobromination of 3(α),12(β)-Diacetoxy-23-bromonorcholane. With Collidine—Exactly 100 mg. of the 23-bromo compound were refluxed for 4½ hours with 10 cc. of freshly distilled collidine. The collidine was removed *in vacuo*, the residue taken up in ether and water, and the ethereal solution washed with water, 10 per cent sulfuric acid, water, 5 per

cent sodium carbonate solution, and finally twice with water. On removal of the ether, a red oil, weighing 88 mg., was obtained. This resisted crystallization. On chromatographic fractionation, 71 mg. of starting material were isolated. No other crystalline compound could be found.

With Piperidine—A solution of 0.22 gm. of the bromide in 5 cc. of anhydrous piperidine was heated in a sealed tube in a bath of boiling *n*-butyl alcohol (117°) for 42 hours. When the solution was cooled in an ice-bath, light, thin plates crystallized. The crystalline material did not dissolve upon addition of ether. The solvents were removed *in vacuo* and the residue taken up in ether and water. The ethereal solution was washed as described above and evaporated; an oily residue weighing 25 mg. was obtained. It may be concluded that the water-soluble piperidinium complex, stable at 117°, was formed by this treatment.

With Alcoholic Silver Nitrate—To a solution of 0.20 gm. of the 23-bromo compound in 95 per cent ethanol were added about 20 cc. of a 5 per cent solution of silver nitrate in ethanol. The solution was refluxed for 10 minutes and the precipitated silver bromide was filtered. From the solution, 20 mg. of impure crystalline material, m.p. 131–136°, were obtained by crystallization from methanol. An attempt was made to purify this by chromatographing, but only 7 mg. of material, melting at 118–121°, resulted. The nature of this compound was not determined because of lack of sufficient material.

With Sodium Ethylate—To a boiling solution of the sodium ethylate prepared from 12.4 gm. of sodium and 148 cc. of absolute ethanol was added a solution of 1.00 gm. of the 23-bromide in 52 cc. of hot absolute ethanol. Refluxing was continued for 30 minutes, and then 100 cc. of water were added. Most of the solvent was removed by distillation and the residue was poured into 1 liter of cold water. This was acidified with sulfuric acid, and the organic material removed by extraction with ether. The ethereal solution was washed with water, 5 per cent sodium carbonate solution, and again with water. The ether was removed, and the residue was dried and reacetylated in the usual manner. The resulting oil weighed 0.88 gm. This was chromatographed over alumina. The only crystalline fraction, eluted by 50 per cent benzene-50 per cent petroleum ether, yielded 50 mg. of material, m.p. 139–144°. The Beilstein test was negative. One recrystallization from methanol gave a product melting at 143–144°. $[\alpha]_D^{20} = +60°$. The analysis is satisfactory for an acetoxy ethoxy norcholene.

Analysis—$C_{27}H_{44}O_3$. Calculated, 77.83, H 10.65; found, C 77.67, H 10.85

Action of Collidine on Isomeric 3(α),12(β)-Diacetoxy-20-bromopregnane Mixture—A solution of 0.10 gm. of the 20-bromide mixture (m.p. 95–102°) in 10 cc. of collidine was refluxed for 2 hours. The solvent was removed *in*

vacuo at 80° and the residue taken up in ether and water. The aqueous washings of the ethereal solution were analyzed for halide ion by Volhard titration. They contained 1.34×10^{-5} mole of bromide, corresponding to only a 6 to 7 per cent reaction.

Action of Piperidine on Isomeric 3(α),12(β)-Diacetoxy-20-bromopregnane Mixture—Exactly 100 mg. of the isomeric 20-bromide mixture (m.p. 95–102°) were refluxed in 5 cc. of anhydrous piperidine for 22 hours. The reaction mixture was worked up in the usual manner, yielding 74 mg. of crystalline residue. This was dissolved in methanol and on cooling gave 50 mg. of crystals, melting at 162–174°. After five recrystallizations from methanol the product showed a constant melting point of 176–178.5°, $[\alpha]_D^{20} = +112°$. The analysis is correct for either 3(α),12(β)-diacetoxy-$\Delta^{17,20}$-pregnene (XIV) or 3(α),12(β)-diacetoxy-$\Delta^{20,21}$-pregnene (XV), or for a mixture of both isomers.

Analysis—$C_{25}H_{38}O_4$. Calculated, C 74.59, H 9.51; found, C 74.90, H 9.66

An additional 20 mg. of the same material were obtained by careful addition of water to the mother liquor.

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SUMMARY

The diacetates of desoxycholic acid, nordesoxycholic acid, and bisnordesoxycholic acid, and the acetate of 3(α)-hydroxy-12-ketocholanic acid have been converted by the action of bromine on their silver salts to steroid bromides containing 1 less carbon atom. The bromo compounds so prepared are 3(α),12(β)-diacetoxy-23-bromonorcholane, m.p. 127.5–128.5°; 3(α),12(β)-diacetoxy-22-bromobisnorcholane, m.p. 144.5–145°; 3(α),12(β)-diacetoxy-20(α)-bromopregnane, m.p. 83–87°; 3(α),12(β)-diacetoxy-20(β)-bromopregnane, m.p. 140–142°; and 3(α)-acetoxy-12-keto-23-bromonorcholane, m.p. 209.5–211°.

These bromides have been reduced to the corresponding compounds with alkyl side chains at C_{17}: 3(α),12(β)-diacetoxynorcholane, m.p. 116.5–117°; 3(α),12(β)-diacetoxybisnorcholane, m.p. 115–116°; 3(α),12(β)-diacetoxypregnane, m.p. 158–158.5°; and 3(α)-acetoxy-12-ketonorcholane, m.p. 158.5–159.5°.

By the action of piperidine, a mixture of the isomeric diacetoxy-20-bromopregnanes has been dehydrobrominated. The product, m.p. 176–

178.5°, is a pregnenediol diacetate with the double bond at C_{17}–C_{20} or at C_{20}–C_{21}, or a mixture of both isomers.

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THE RELATIONSHIP BETWEEN OPTICAL ROTATORY POWER AND CONSTITUTION OF THE STEROLS. III

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In the previous two papers of this series (1) the application of the modern theories of optical rotatory power to steroids was discussed, and a method of calculation of the rotatory power of these compounds was developed. It was shown, for example, that α-steroids of the cholestanol type will always have a molecular rotation greater by 2540° ($CHCl_3$), in the positive direction, than the corresponding β-form. In the coprostanol series the α-form will always be higher by 2680°. It is our purpose in this paper to present evidence which shows that configurations can be assigned to diastereoisomers at the C_3 position of many other steroids from a knowledge of their optical rotatory powers. By these additional correlations, the validity of the assumptions made in our calculations (1) becomes more firmly established.

In this connection we have amplified the work of Callow and Young (2) who made a study of the relationship between optical rotatory power and the constitution of steroids. Among other things, these investigators pointed out that in fifteen out of eighteen cases an increase in the dextrorotatory power results from inversion of the C_3—OH group from the β- to the α-position. We have listed in Table V eighty-two pairs of compounds epimeric at the C_3 position with their melting points and, where known (fifty-seven pairs), optical rotatory powers. An analysis of these optical data has been made in Tables II, III, and IV and allows the following conclusion to be made:

The C_3 α-form of any steroid will have a higher positive rotatory power (sodium D light) than the corresponding β-form *regardless of the solvent used.*

We would like to point out that the only exceptions to this rule may be in its application to $\Delta^{5:6}$-stenols in solvents other than chloroform.

In fifty-one out of fifty-four cases ($\Delta^{5:6}$-stenols in alcohol are excluded) the α-form has the higher positive rotatory power. This fact corroborates, then, the basic assumptions made by us in calculating the rotatory powers of steroids. We are of the opinion that the three exceptions are in error which may be ascribed to the purity of the compounds involved or that an error was made in the determination of their rotations. Moreover, the chemist now has an additional method for assigning configurations to C_3-diastereomers purely on the basis of optical rotatory power.

We have also made a thorough study of the melting point relationship of α and β-diastereoisomers. Previous work on this subject may be found in the literature. Ruzicka, Wirz, and Meyer (3) have assumed on the basis of α-cholestanol

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having a higher melting point than β-cholestanol that the same melting point relationship would hold for the corresponding chlorides. Also Reindel and

TABLE I

(Melting Point)

CLASS OF COMPOUND	NO. OF C_3-EPIMERS IN WHICH THE α-FORM HAS A HIGHER M.P.	NO. OF C_3-EPIMERS IN WHICH THE β-FORM HAS A HIGHER M.P.
Saturated sterols	7	0
Saturated sterol derivatives	2	6
Unsaturated sterols	5	7
Unsaturated sterol derivatives	1	16
Bile acids	3	3
Bile acid methyl esters	2	3
Derivatives of bile acids or methyl ester	4	4
"Pregnane" compounds	2	1
"Pregnane" derivatives	0	2
Unsaturated "pregnane" compounds	1	1
Unsaturated "pregnane" derivatives	0	1
"Androstane" compounds	3	1
"Androstane" derivatives	1	0
Unsaturated "androstane" compounds	2	0
Unsaturated "androstane" derivatives	1	0
"Cardiac aglucon" derivative	1	0

TABLE II

(Rotations: $CHCl_3$)

CLASS OF COMPOUND	NO. OF C_3-EPIMERS IN WHICH α-FORM HAS A HIGHER POSITIVE $[\alpha]_D$	NO. OF C_3-EPIMERS IN WHICH β-FORM HAS HIGHER POSITIVE $[\alpha]_D$
Saturated sterols	3	1
Saturated sterol derivatives	2	0
Unsaturated sterols	7	0
Unsaturated sterol derivatives	10	1
Bile acids	2	0
Bile acid derivatives	1	0
"Cardiac aglucon" derivative	1	0

Niederländer (4) have made a comparison between the melting points of a large number of saturated stereoisomers. In both the cholestane and coprostane series, the member of an epimeric pair that gives an insoluble digitonide (*i.e.*, β-form) nearly always has the lower melting point.

However, Marker *et al.* (5) have pointed out in $\Delta^{5:6}$-stenols that the β-form has the higher melting point. Landenburg, Chakravorty, and Wallis (6) have also noted that application of the assumption that the α-forms have higher melting

TABLE III

(Rotations: Alcohol or Absolute Alcohol)

Class of compound	No. of C_3-epimers in which α-form has higher positive $[\alpha]_D$	No. of C_3-epimers in which β-form has higher positive $[\alpha]_D$
Saturated sterols	1	0
Unsaturated sterols ($\Delta^{5:6}$)	0	1
Bile acids	3	0
"Pregnane" compounds	2	1
"Pregnane" derivatives	2	0
Unsaturated "pregnane" compounds ($\Delta^{5:6}$)	1	0
Unsaturated "pregnane" compounds ($\Delta^{17:20}$)	1	0
Unsaturated "pregnane" derivatives ($\Delta^{5:6}$)	1	0
"Androstane" compounds	1	0
Unsaturated "androstane" derivatives ($\Delta^{5:6}$)	0	2

TABLE IV

(Rotations: Miscellaneous Solvents)

Class of compound	No. of C_3-epimers in which α-form has higher positive $[\alpha]_D$	No. of C_3-epimers in which β-form has higher positive $[\alpha]_D$
Unsaturated sterols	1 (B)	0
Bile acid methyl ester	1 (D)	0
	2 (Ac)	0
Bile acid methyl ester derivatives	7 (Ac)	0
"Androstane" compounds	2 (M)	0
	1 (H)	0
"Androstane" derivatives	1 (M)	0

Ac = acetone; B = benzene; D = dioxane; H = 1 *N* acetic acid; M = methyl alcohol.

points than the corresponding β-forms leads to confusion in assigning configurations to the 3-cholorocholestanone-6's.

An analysis of the melting point data in Table V has been made in Table I, and the following conclusions can be made:

1. The C_3 α-form of an unsubstituted saturated sterol will have a higher melting point than the corresponding β-form.

TABLE V

COMPOUND	C_3 CONFIGURATION	M.P. °C	$[\alpha]_D$	SOLVENT[a]	REF.
A. Saturated Sterols					
1. Cholestanol	β	140–141	+23	C	7
			+29	A	8
	α	182–184	+32.2	C	7
			+34	A	8
2. Stigmastanol	β	136–137	+23.8	C	9
	α	203	+26.0	C	10
3. Ergostanol	β	141	+15.3	C	11
	α	205	+14.6	C	12
4. Coprostanol	β	101–102	+23.6	C	13
	α	116–118	+31.6	C	13
5. Norcoprostanol	β	117–118			4
	α	153–154	(+29.2	B)	14
6. Bisnorcoprostanol	β	126–127			4
	α	134–135			4
7. 24-Ethylcoprostanol	β	127			15
	α	137			15
B. Saturated Sterol Derivatives					
1. Cholestanyl acetate	β	109–110	(+11.5	C)	16
	α	95.5–96			17
2. Stigmastanyl acetate	β	129–129.5	+15.4	C	9
	α	88	+28.0	C	10
3. Ergostanyl acetate	β	143–144	+6.3	C	11
	α	144	+20.5	C	12
4. Coprostanyl acetate	β	88–89			18
	α	87–88	(+43.8	B)	14
5. Norcoprostanyl acetate	β	122–123			4
	α	93–94	(+48.2	B)	14
6. Bisnorcoprostanyl acetate	β	103–104			4
	α	93–94			4
7. 24-Ethylcoprostanyl acetate	β	89			15
	α	94			15

TABLE V—*Continued*

COMPOUND	C_3 CONFIGURATION	M.P. °C	$[\alpha]_D$	SOLVENT[a]	REF.
8. Coprostanyl benzoate	β	114–115			19
	α	85–86			20
C. Unsaturated sterols					
1. Allocholesterol	β	132	+43.7	B	21
	α	84	+120.8	B	21
2. Allo-β-sitosterol	β	158			22
	α	138			22
3. Cholesterol	β		−38.8	C	23
		147–149	−31.0	A	24
	α	140.5	−34	C	7
			−37.5	A	24
4. β-Sitosterol	β	136–137	(−36.6	C)	25
	α	135			5
5. Stigmasterol	β	168–170	(−51.0	C)	26
	α	151			5
6. 7-Dehydrocholesterol	β	142–143.5	−113.6	C	27
	α	124–126	−70.5	C	28
7. Allodehydrocholesterol	β	115–116	+10	C	29
	α	93–94	+80	C	29
8. Zymosterol	β	108–110	+49	C	30
	α	160–162	+55	C	31
9. Dihydrozymosterol	β	128–129	+50	C	30
	α	183	+56	C	31
10. Ergosterol-D	β	165–166	+22.0	C	32
	α	203–204	+36.2	C	33
11. Neoergosterol	β	152	−12	C (?)	34
	α	177	+28.7 av.	C	35
12. 22-Dihydroneo-ergosterol	β	150			36
	α	167	(+28.8	C)	35
D Unsaturated sterol derivatives					
1. Allocholesteryl acetate	β	85			21
	α	82.5			21

TABEL V—*Continued*

COMPOUND	C_3 CONFIGURATION	M.P.°C	$[\alpha]_D$	SOLVENT[a]	REF.
2. Allo-β-sitosteryl acetate	β	88			22
	α	92			22
3. Cholesteryl acetate	β	114.7–115.6	(−42.0	C)	23
	α	85			24
4. β-Sitosteryl acetate	β	125–126	(−41.0	C)	25
	α	66			5
5. Stigmasteryl acetate	β	143–144	(−55.6	C)	26
	α	98			5
6. 7-Dehydrocholesteryl-acetate	β	130	−77.6 (calc'd)	C	m.p. 37 rot. 38
	α	114–115	−35	C	28
7. Allodehydrocholesterylacetate	β	109	−56.0	C	29
	α	96	+126.3	C	29
8. Zymosteryl acetate	β	106–108	(+34	C)	30
	α	83–85			31
9. Dihydrozymosteryl acetate	β	128–129	+31.5	C	30
	α	85–87	+40	C	31
10. Ergosteryl-D acetate	β	173–174	+20.7	C	33
	α	150	+40.6	C	33
11. Neoergosteryl acetate	β	122–123	−8	C	m.p. 34 rot. 39
	α	98	+27.2	C	
12. 22-Dihydroneoergosteryl acetate	β	118	−3.1	C	36
	α	83	+24.6	C	35
13. Cholesteryl benzoate	β	148–50 and 177	−17.0	C	40
	α	99.5	−29	C	7
14. 7-Dehydrocholesteryl benzoate	β	139–40 and 183	−53.2	C	27
	α	118–119	+48.5	C	28
15. Allodehydrocholesteryl *m*-dinitrobenzoate	β	154(180–185)	−78.5	C	29
	α	150(180–185)	+159	C	29
16. Neoergosteryl *m*-dinitrobenzoate	β	218–220	−13	C	41
	α	204	+21.2	C	35

TABLE V—*Continued*

COMPOUND	C_3 CONFIGURATION	M.P. °C	$[\alpha]_D$	SOLVENT[a]	REF.
17. Neoergosteryl methyl ether	β	94	−5	C	35
	α	74	+18.4	C	35
E. Bile Acids					
1. 3-Hydroxycholanic acid	β	179	+25.1	A	8
	α	188	+33.6	A	8
2. 3-Hydroxyallocholanic acid	β	218	+17.2	C	m.p. 42 rot. 43
	α	208–210	+23.3	C	m.p. 44 rot. 43
3. 3-Hydroxynorallocholanic acid	β	226			42
	α	205–207	(+21.0	C)	10
4. 3-Hydroxybisnorallocholanic acid	β		−10.1 (calc'd)	C	38
	α	220	+17	C	10
5. 3-Hydroxy-12-ketocholanic acid	β	218–220	+90.5	A.A.	45
	α	164–165	+110.2	A.A.	45
6. Hyodesoxycholic acid	β	189–190	+5.1	A	46
	α	196–197	+8.4	A (?)	m.p. 47 rot. 46
7. 3-Hydroxy-11-cholenic acid	β	*ca.* 128	+27.8	D	48
	α	165–166	+33.2	D (?)	48
F. Bile acid methyl esters					
1. Methyl 3-hydroxyallocholanate	β	151	(+18.4	A)	44
	α	164	(+17.7	C)	44
2. Methyl 3-hydroxynorallocholanate	β	156			42
	α	169–170	(+21.0	C)	10
3. Methyl 3-hydroxy-11β, 12β-oxidocholanate	β	114–115	+27.1	Ac	48
	α	96–98	+35.7	Ac	48
4. Methyl 3-hydroxy-11-ketoetiocholanate	β	172–175	+72.1	Ac	49
	α	155–158			49
5. Methyl 3-hydroxy-11-etiocholenate	β	131–133	+70.7	Ac	50
	α	122–124	+77.7	Ac	50
G. Derivatives of bile acids or methyl esters					
1. 3-Acetoxybisnorallocholanic acid	β		−19.5 (calc'd)	C	38
	α	225–227	+2	C	10

TABLE V—*Continued*

COMPOUND	C_3 CONFIGURATION	M.P. °C	$[\alpha]_D$	SOLVENT[a]	REF.
2. Methyl 3-acetoxynorallocholanate	β	163			42
	α	189–190	(+26.0	C)	10
3. Methyl 3-acetoxy, 11β, 12β-oxido-cholanate	β	150–152	+31.2	Ac	48
	α	140–142	+52.8	Ac	48
4. Methyl 3-acetoxy-11-ketocholanate	β	173–174	+56.4	Ac	51
	α	132–133	+67.1	Ac	52
5. Methyl 3-acetoxy-11-etiocholenate	β	70–72	+62.5	Ac	50
	α	99–100	+87.7	Ac	50
6. Methyl 3-acetoxy-12-keto-9-cholenate	β	192–193	+73.9	Ac	51
	α	149–150	+102.5	Ac	52
7. Methyl 3-acetoxy-11α-hydroxycholanate	β	139–140	+50.0	Ac	51
	α	146–148	+70.7	Ac	52
8. Methyl 3-acetoxy-12-ketocholanate	β	184–186	+77.9	Ac	51
	α	153.5–154.5	+104.8	Ac	53
9. Methyl-3-acetoxy-11-ketoetiocholanate	β	129–131	+71.8	Ac	49
	α	147–149	+98.1	Ac	49
H. "Pregnane" compounds					
1. Allopregnan-ol-3-one-20	β	194	+90.5	A.A.	54
	α	176–178	+87.7	A.A.	54
2. Pregnan-ol-3-one-20	β	142–143	+101.6	A.A.	55
	α	148–149	+113.8	A.A.	55
3. 20-Methylpregnanediol-3,20	β	168–171	+16.5	A.A	55
	α	190–201	+23.0	A.A.(?)	55
I. "Pregnane" derivatives					
1. Allopregnanol-3-one-20 acetate	β	144	+79.8	A.A.	54
	α	141–142	+94.5	A.A.	54
2. Pregnanol-3-one-20 acetate	β	116.5	+86.5	A.A.	55
	α	99	+123.7	A.A.	55
J. Unsaturated "pregnane" compounds					
1. Δ^5-Pregnenol-3-one-20	β	189–190	+30	A	56
	α	148–152	+54.5	A	57
2. 20-Methyl-$\Delta^{17;20}$-pregnenol-3	β	141–142	+14.7	A.A.	55
	α	164–165.5	+45.4	A.A.(?)	55

TABLE V—*Concluded*

COMPOUND	C_3 CONFIGURATION	M.P. °C	$[\alpha]_D$	SOLVENT[a]	REF.
K. Unsaturated "pregnane" derivatives					
1. Δ^5-Pregnenolone acetate	β	149–150	+22	A	58
	α	147	+57.2	A (?)	57
L. "Androstane" compounds					
1. Androstanediol-3,17 (α)	β	164	+4.2	A (?)	59
	α	221	+12.6	A (?)	66
2. Androstan-ol-3-one-17	β	172	+88.6	M	61
	α	182–183	+103.5	M	62
3. 3-Hydroxy-D-homoandrostanone-(17a)	β	193–195	−66.5	M	63
	α	203–205	−35.5	M	63
4. 3,17-Dihydroxy-17-aminoethylandrostane	β	222–225	−16.5	H	63
	α	204–206	+4.5	H	63
M. "Androstane" derivatives					
1. 3-Acetoxy-D-homoandrostanone (17a)	β	124–125	−45	M	63
	α	150–151	−21.7	M	63
N. Unsaturated "androstane" compounds					
1. Dehydroandrosterone	β	148	+10.9	A	64
	α	221	0	A	65
2. Δ^5-Androstenediol-3,17 (α)	β	182–183	−49.4	A	m.p. 65 rot. 66
	α	208–209	−54	A	
O. Unsaturated "androstane" derivatives					
1. Dehydroandrosterone acetate	β	168–169	(+3.9	A)	64
		171–172			67
	α	173–174.5			24
P. "Cardiac aglucon" derivative					
$\Delta^{20:22}$-3-Acetoxy-21-hydroxynorallocholenic acid lactone	β	193–194	+1	C	68
	α	230	+19	C	68

[a] C = chloroform.
A = alcohol.
A.A. = absolute alcohol.
B = benzene.
Ac = acetone.
D = dioxane.
M = methyl alcohol.
H = 1 *N* acetic acid.

2. Application of this rule to other steroids is unwarranted.

3. The rule is most likely reversed for $\Delta^{5:6}$-stenols and for certain derivatives of unsaturated sterols.

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